

1994020886

**N94- 25368**

**DESIGN AND TESTING OF A UNIQUE  
RANDOMIZED GRAVITY,  
CONTINUOUS FLOW BIOREACTOR**

**Final Report  
NASA/ASEE Summer Faculty Fellowship Program--1993  
Johnson Space Center**

Prepared By:	Carroll B. Lassiter, Ph.D.
Academic Rank:	Associate Professor
University and Department	University of Houston- Clear Lake Division of Biology Houston, Texas 77058
NASA/JSC	
Directorate:	Space and Life Sciences
Division:	Medical Sciences
Branch:	Biomedical Laboratories
JSC Colleague:	Duane L. Pierson, Ph.D.
Date Submitted:	October 6, 1993
Contract Number:	NGT-44-001-800

(VOLUME 2)

## ABSTRACT

A rotating, null gravity simulator, or Couette bioreactor has been successfully used for the culture of mammalian cells in a simulated microgravity environment. Two limited studies using Lipomyces starkeyi and Streptomyces clavuligerus were also conducted under conditions of simulated weightlessness. Although these studies with microorganisms showed promising preliminary results, oxygen limitations presented significant limitations in studying the biochemical and cultural characteristics of these cell types. Microbial cell systems such as bacteria and yeast promise significant potential as investigative models to study the effects of micro gravity on membrane transport, as well as substrate induction of inactive enzyme systems. Additionally, the smaller size of the microorganisms should further reduce the gravity induced oscillatory particle motion and thereby improve the microgravity simulation on earth. This project focuses on the unique conceptual design, and subsequent development of a rotating bioreactor that is compatible with the culture and investigation of microgravity effects on microbial systems. The new reactor design will allow testing of highly aerobic cell types under simulated microgravity conditions. The described reactor affords a mechanism for investigating the long term effects of reduced gravity on cellular respiration, membrane transfer, ion exchange and substrate conversions. It offers the capability of dynamically altering nutrients, oxygenation, pH, carbon dioxide and substrate concentration without disturbing the microgravity simulation, or Couette flow, of the reactor. All progeny of the original cell inoculum may be acclimated to the simulated microgravity in the absence of a substrate or nutrient. The reactor has the promise of allowing scientists to probe the long term effects of weightlessness on cell interactions in plants, bacteria, yeast and fungi. The reactor is designed to have a flow field growth chamber with uniform shear stress, yet transfer high concentrations of oxygen into the culture medium. The system described allows for continuous, on line sampling for production of product without disturbing fluid and particle dynamics in the reaction chamber. It provides for the introduction of substrate, or control substances after cell adaptation to simulated microgravity has been accomplished. The reactor system provides for the non disruptive, continuous flow replacement of nutrient and removal of product. On line monitoring and control of growth conditions such as pH and nutrient status are provided. A rotating distribution valve allows for adjustment and alterations of all parameters without cessation of growth chamber rotation, thereby preserving the simulated microgravity conditions over longer periods of time.

## INTRODUCTION

Clinostats have been developed and used to study higher plant growth in a simulated micro gravity for a number of years (1). Likewise, a null gravity simulator, or couette bioreactor has been developed and used for culture of mammalian cells in a simulated microgravity environment. Although a great deal of attention is being given to the culture of mammalian cells in a simulated micro environment, little has been done to study gravitational effect on the biochemical and cultural characteristics of microorganisms such as bacteria and fungi. Limited studies growing microorganisms in simulated weightlessness showed promising preliminary results, however oxygen limitations present problems in studying the biochemical and cultural characteristics of these microorganisms.

Cellular systems found in microbes such as bacteria, fungi and yeasts offer significant potential as investigative models for studying the effects of microgravity on membrane transport and substrate induction of inactive metabolic pathways. Additionally, because of extensive chromosomal mapping in some organisms, one can learn a great deal about mediator effects on the molecular biology of the genome.

It can be hypothesized that the smaller size of the microorganisms will reduce the gravity induced oscillatory particle motion in a couette flow bioreactor (1). It has been shown that as cell aggregates increase in size, the oscillation of the particle deviates from the ideal in a direct correlation. This suggests that the smaller size of microorganisms, and their lack of aggregation in a couette flow bioreactor would produce less gravity induced oscillation than found in mammalian cell aggregates being cultured.

The focus of this project was to develop a conceptual design and model of a rotating bioreactor that will support ideal growth conditions for microbial cell systems growing in a simulated microgravity environment. The system designed was conceived to meet, as a minimum, the following experimental provisions:

- 1.) Couette flow field growth chamber should present uniform shear stress.
- 2.) Oxygen must pass into the culture medium with a high transfer rate.
- 3.) System should allow for continuous, on line sampling of cell products without disturbance of fluid and particle dynamics in the cell growth chamber.
- 4.) The bioreactor should allow for the introduction of substrate, or control mediators, after the cell population has developed in simulated a microgravity environment.
- 5.) It should provide non disruptive, continuous flow replacement of nutrient.

- 6.) It should provide for on line adjustment of the pH of the culture media in order to maintain ideal growth conditions for a given system.

#### DESCRIPTION OF BIOREACTOR DESIGN

The overall flow schematic of the operating bioreactor, as conceived by the author, is shown in Figure 1.. The growth chamber of the bioreactor will be coupled to a standard bench top fermenter such as those presently being used for a wide variety of cell culture. The membrane diffusion chambers are coupled, in parallel to a roller pump, installed in the suction line, leading from the fermenter. This arrangement allows for the standard adjustment of growth conditions in the fermenter, without disturbing the cells in the growth chamber. It should be pointed out that the side diffusion chambers have been depicted much deeper, in relation to the growth chamber, than they really are. Figure 2. is an accurate representation of the size relationships between the three chambers. The thin diffusion chamber design will allow for a short residence time of media in the diffusion chamber. This will assure that the concentration gradients of nutrients, oxygen, and products will be maximized across the semipermeable membranes of the reactor. It is proposed that the bench top fermenter will be 4-6 liters in size, thereby assuring essentially infinite gradients, when interfaced with the significantly smaller bioreactor.

A detailed view of the bioreactor assembly is presented in Figure 3.. It should be pointed out that although dimensions are not presented, the reactor is designed around a 47 mm, off the shelf, general purpose, hydrophilic membrane such as those produced by a number of companies. The membranes are supported by a standard, stainless steel membrane support that is also off the shelf. The chamber provides for easy replacement of membranes and cleaning, since it is sealed by standard o-rings.

Since the bioreactor will be turning on it's central axis, it is necessary to provide for the distribution of fluid from the standard fermenter, while rotation occurs. This distribution is provided for by the rotating fluid distribution valve shown in Figure 4.. Although the valve depicted in this drawing provides for in and out distribution of a single liquid, it could easily be adapted for distribution of multiple fluids by increasing the number of grooves, or by stacking more than one valve assembly.

For compactness and simplicity, the distribution valve has been integrated into the reaction vessel compression and clamping assembly as shown in Figure 5.. This assembly provides uniform pressure on the o-ring seals in the fluid distribution valve by adjusting the compression of the tension spring. Additionally, it provides uniform sealing pressure on

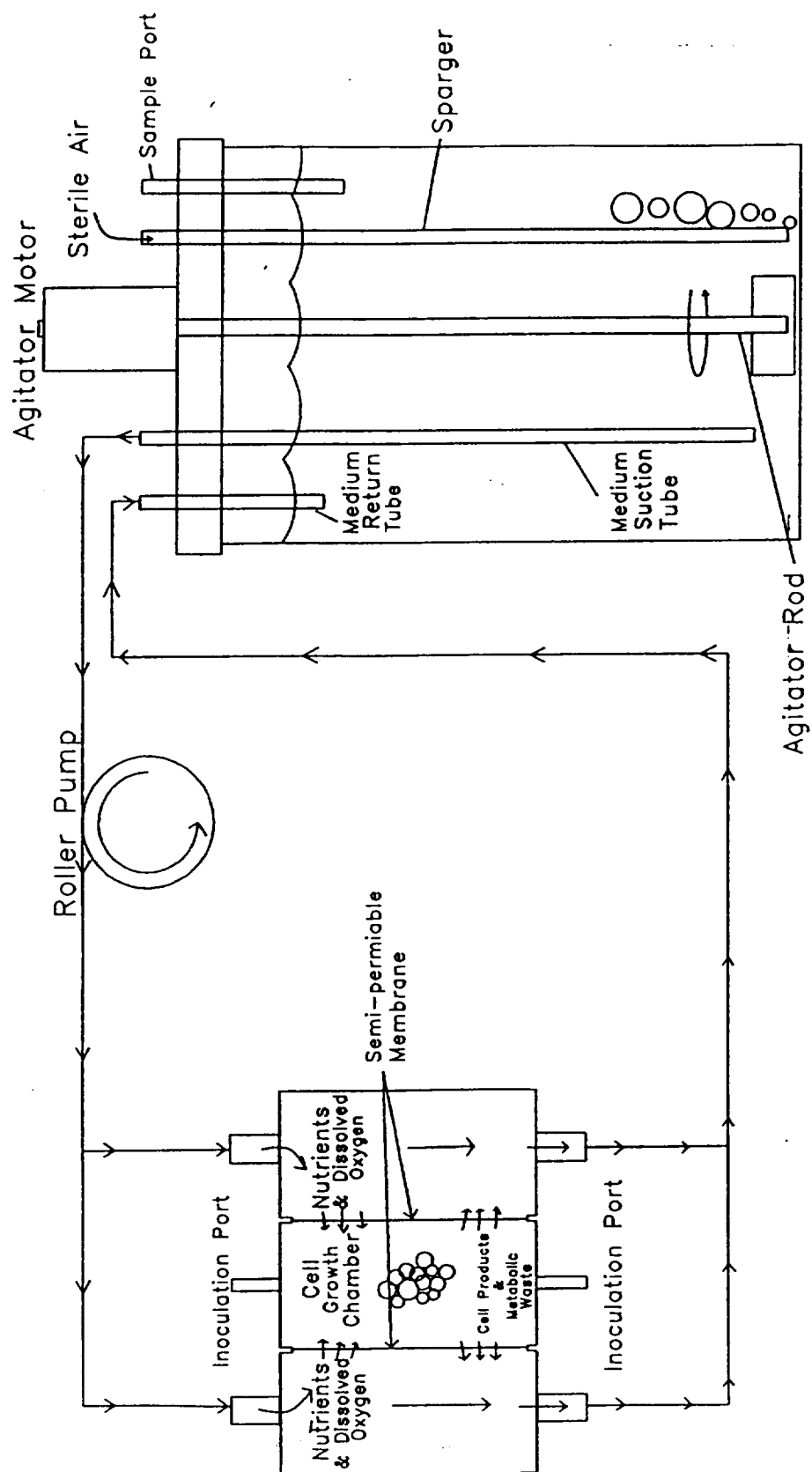


Figure 1. - General operational schematic

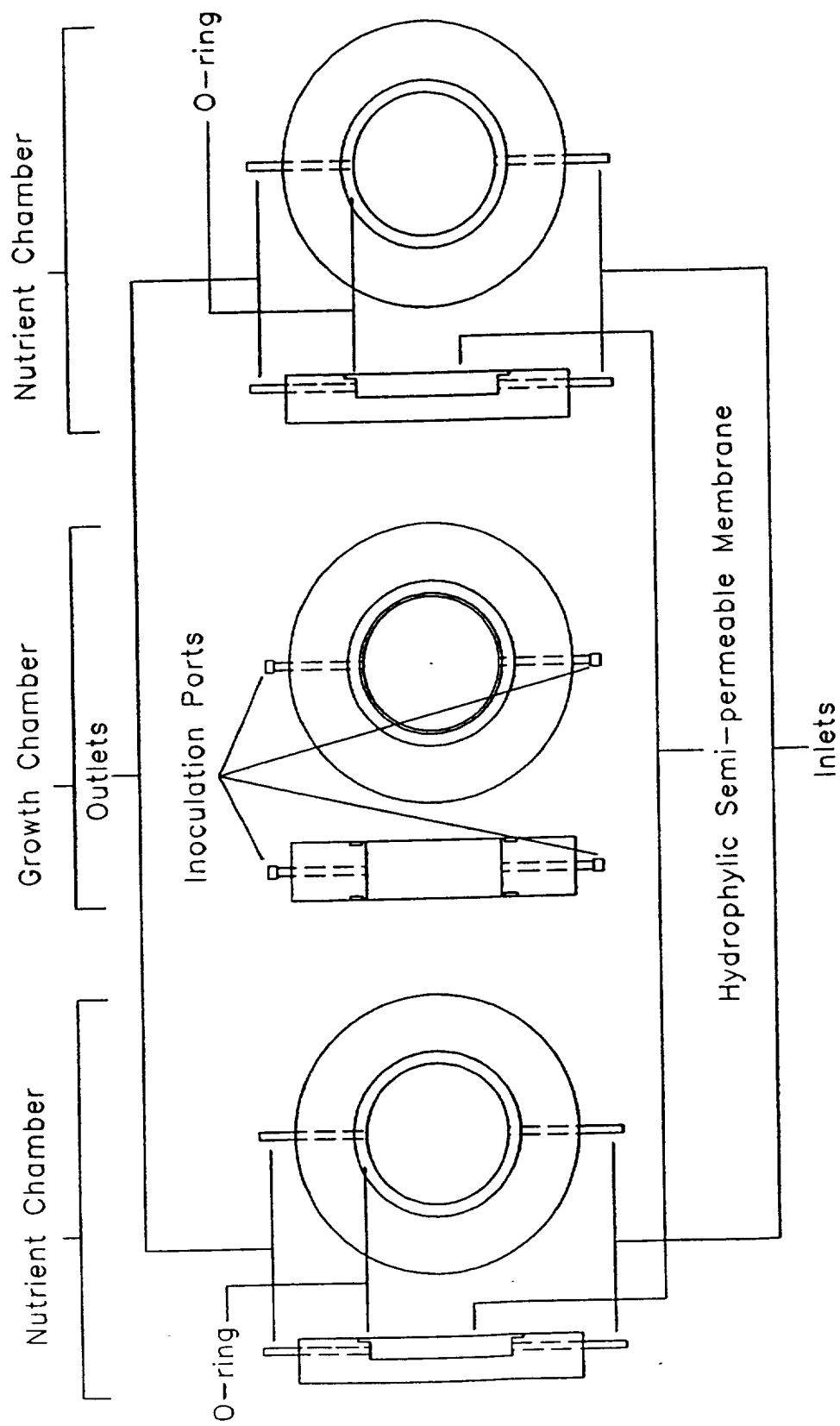
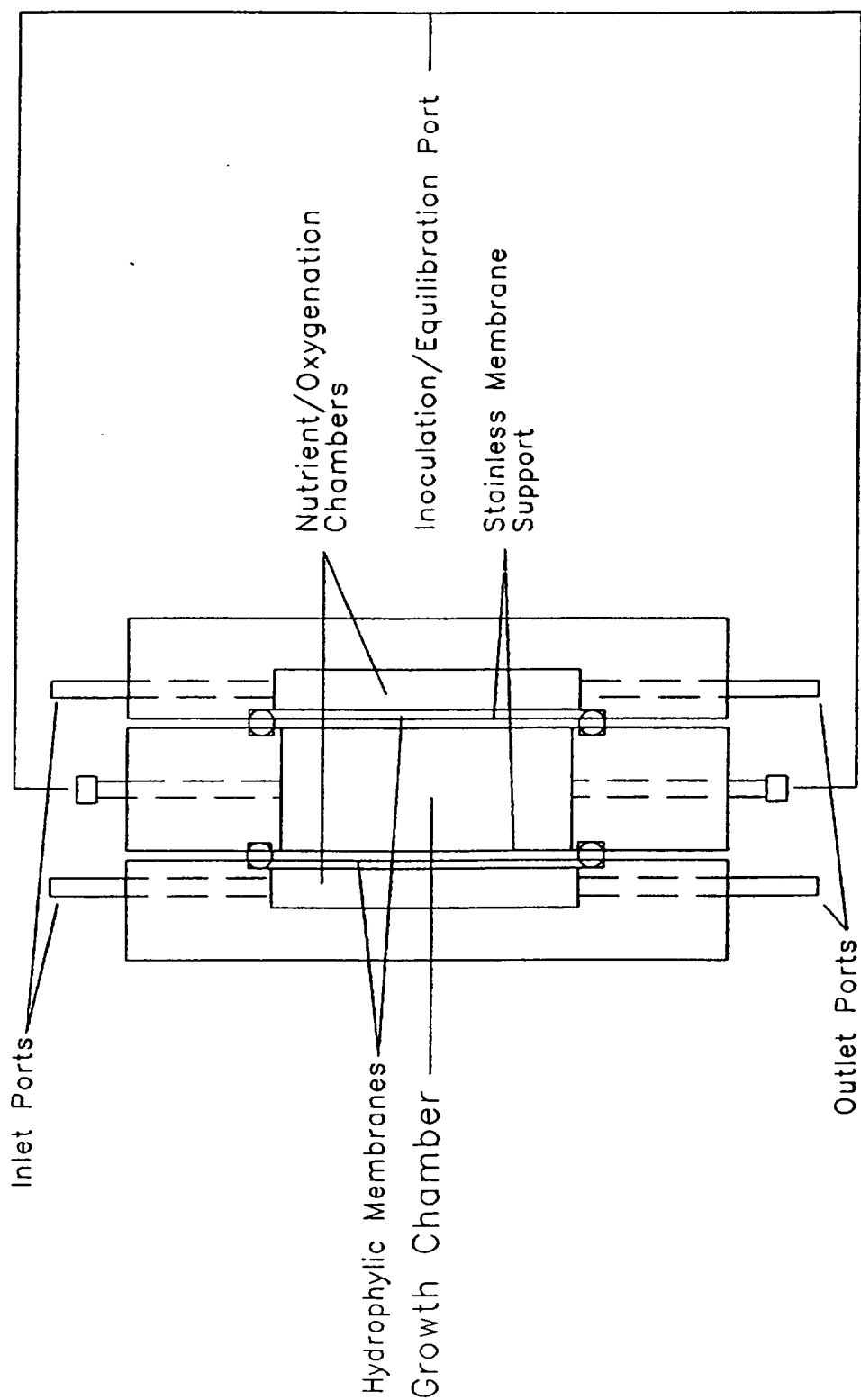


Figure 2. - Diffusion and growth chambers



**Figure 3. - Bioreactor assembly**

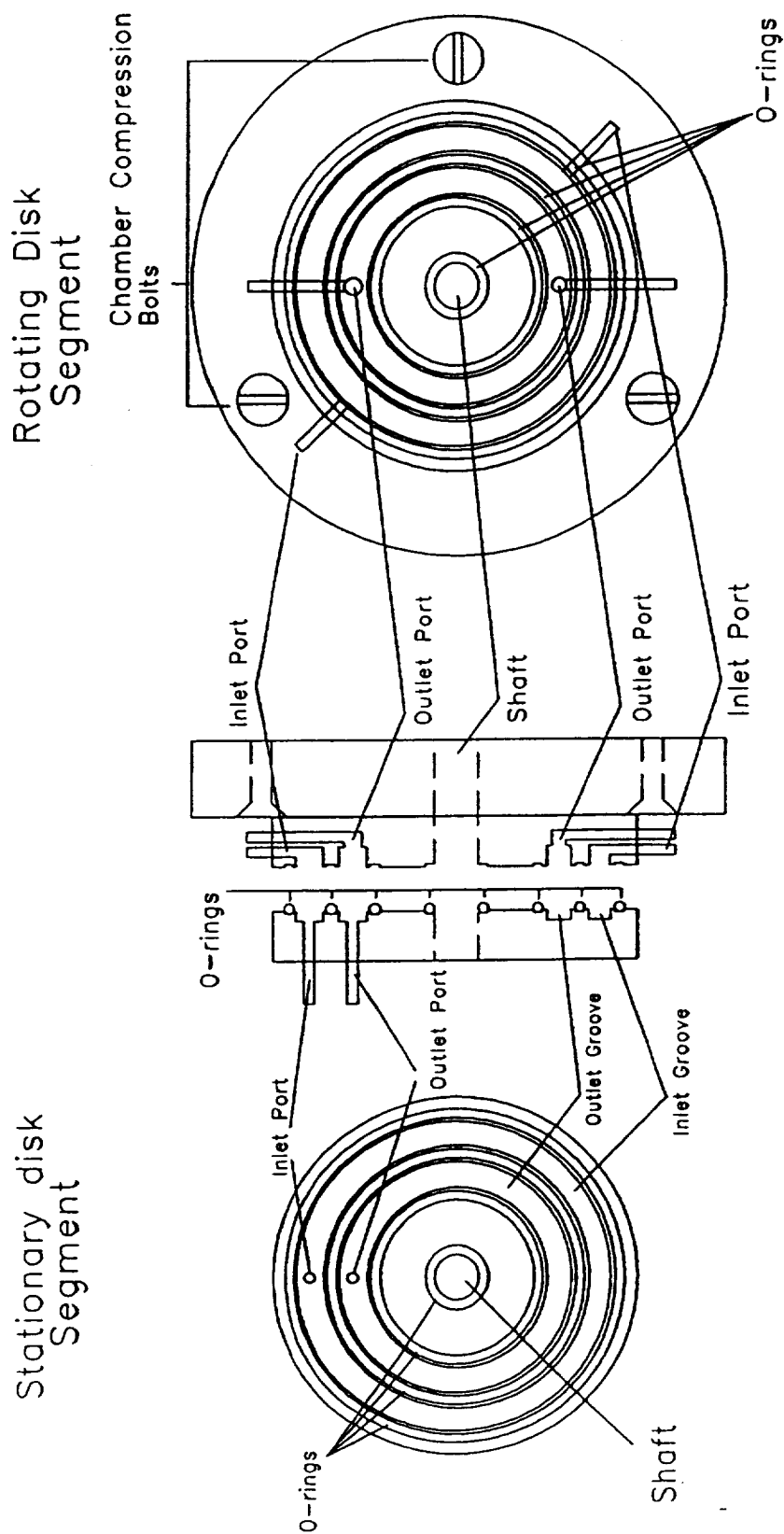
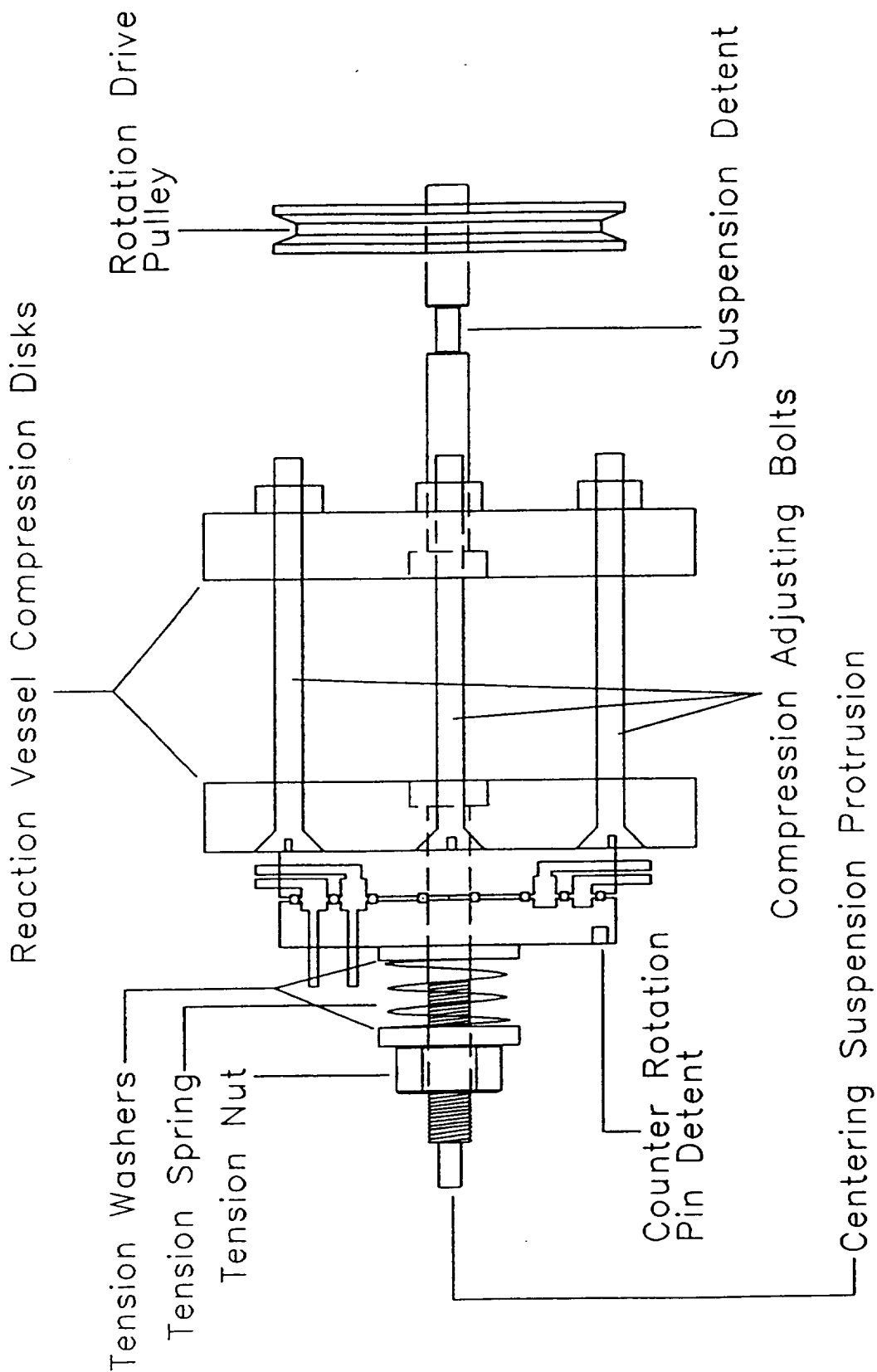


Figure 4. - Rotating fluid distribution valve





**Figure 5. - Reaction vessel compression and clamping assembly**

the o-ring seal in the reaction chamber by adjusting the compression of the reaction vessel compression disks. It is envisioned that the compression bolts will be tightened with a standard torque wrench, measuring inch-ounces of torque.

A schematic of the assembled, operational bioreactor is shown in Figure 6.. The integrated spacial relationships of the growth chamber, fluid distribution valve, drive pulley and clamping assembly are easily visible. For clarity the flows, to and from, the standardized bench-top fermenter are not shown, therefor it should be pointed out that the two lines emerging in the lower left corner are inlet and outlet lines coming from the fermenter.

The support cradle and drive motor for the complete chamber are shown in Figure 7.. For purposes of clarity the large rubber o-ring that is utilized as a drive belt is not shown. The drive belt itself offers sufficient tension to keep the bioreactor in the bearings of the cradle. It should be pointed out that simplicity of design has been paramount in all concepts of the proposed bioreactor. In review of the proposed design, one should find that:

- 1.) Critical items such as membranes, membrane supports, and o-rings would be off the shelf items.
- 2.) The bioreactor can be easily removed from the support cradle for manipulation, when required.
- 3.) The growth chamber and diffusion chambers are easily taken apart and reassembled during cleaning and sterilization.
- 4.) Although the conceptual model described is designed around 47 mm semipermeable membranes, the size could easily be enlarged or decreased, as required for a specific application.

#### SUMMARY

The proposed reactor, coupled with a bench top fermenter via a standard roller pump should allow for testing of highly aerobic cell types under simulated microgravity conditions. Since the oxygen is dissolved in the culture medium at the fermenter, the bioreactor system will require a single hydrophilic membrane type. The described reactor could afford a mechanism of investigating the long term effects of reduced gravity on cellular respiration, membrane transfer characteristic, and genetic induction by molecular mediators. It offers the capability of dynamically altering nutrients, oxygenation, pH, carbon dioxide and substrate concentrations without disturbing the couette flow of the reactor. The diffusion pressure and flows into the reaction chamber are essentially offsetting. The diffusion chambers are equal in all instances, on both sides of the growth chamber. All progeny of the original cell inoculum may be acclimated to the simulated microgravity in the absence of a substrate, nutrient

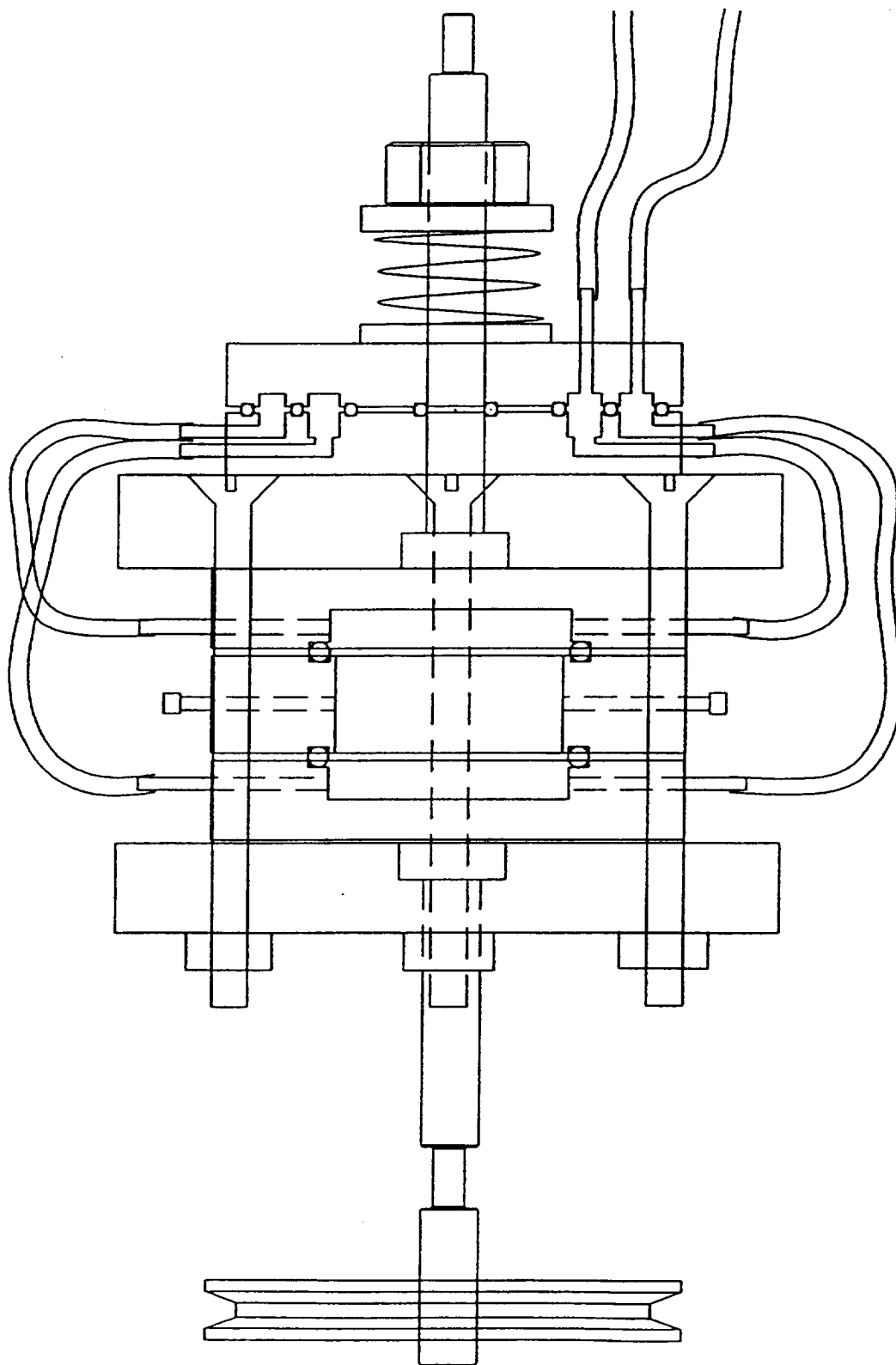


Figure 6. - Operational bioreactor assembly

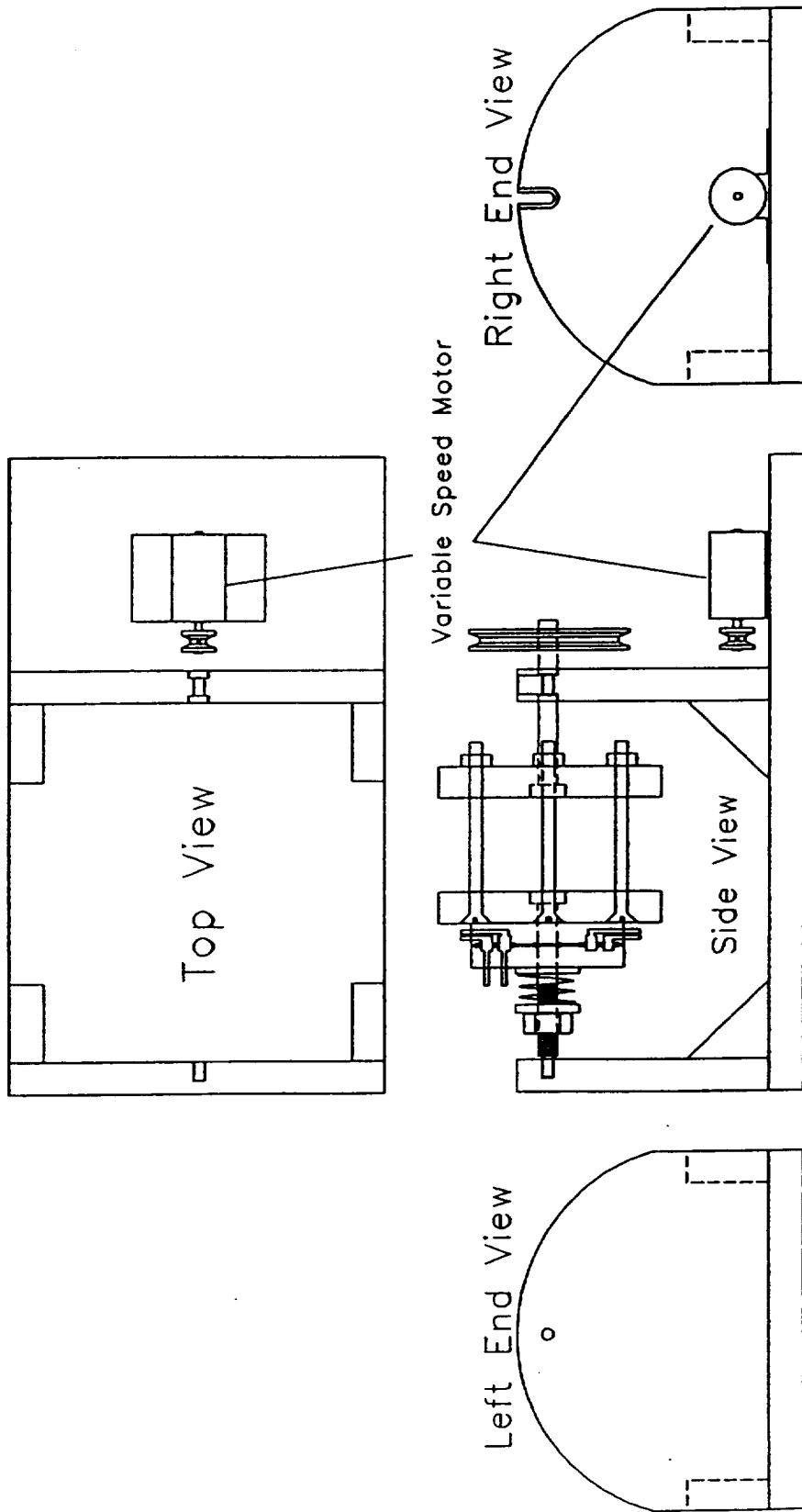


Figure 7. - Support cradle and drive motor

or control substance, whereby "batch" cultures require the addition of these reagents, or potential modifiers, at start up. Although the conceptual model on which these drawings are constructed was completed during the authors NASA/ASEE Summer Faculty Fellowship the operational prototype was not completed. Completion of the prototype is continuing at the University of Houston Clear Lake in cooperation with the NASA/JSC scientists.

A three dimensional representation of the assembled bioreactor is shown in Figure 8 to better illustrate the proposed system.

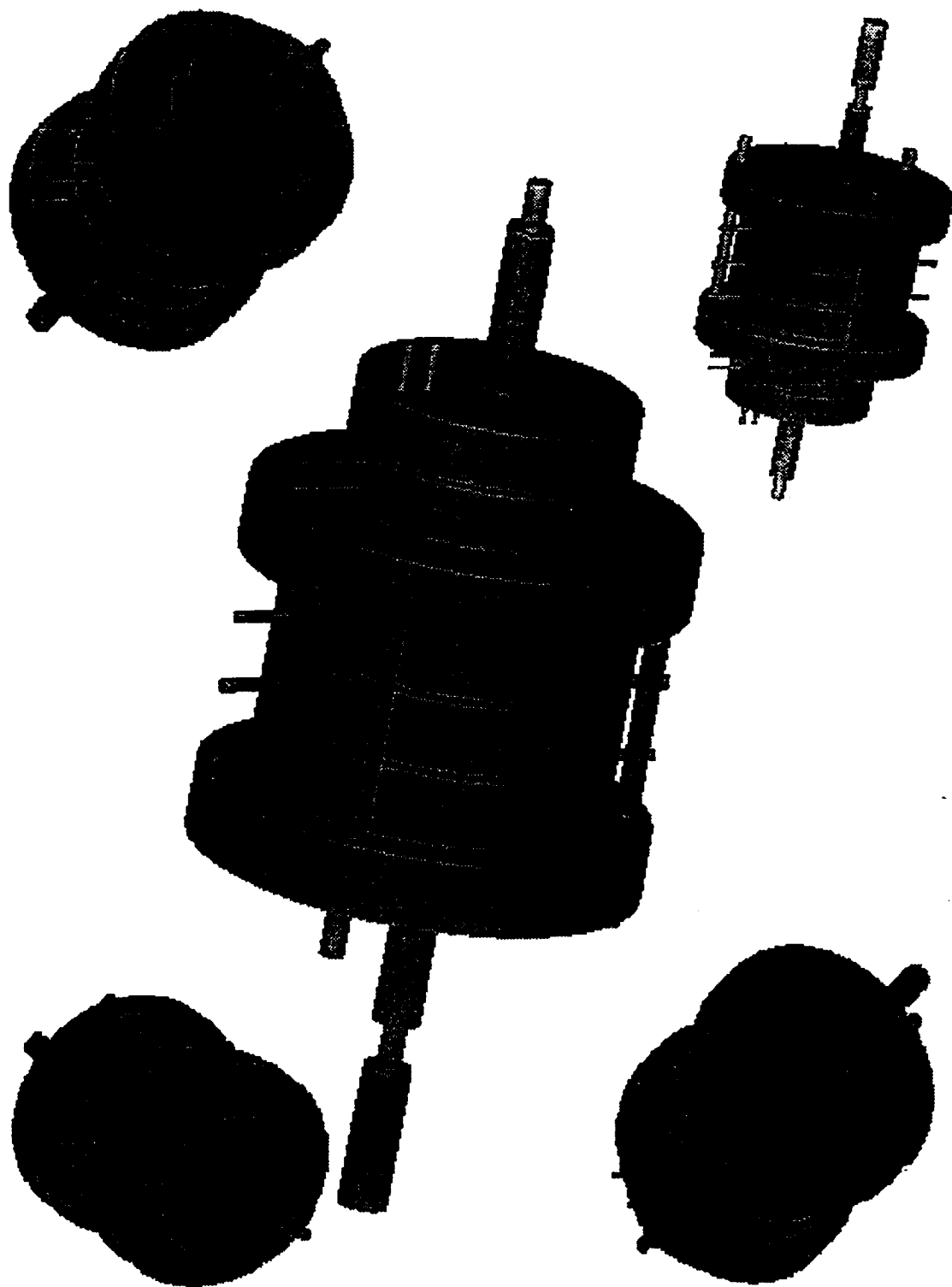


Figure 8. - Three dimensional views of operational bioreactor

#### REFERENCES

1. Halstead, T.W., Todd, P. and Powers, J.B., Editors:  
American Society for Gravitational and Space Biology.  
October, 1992, Volume 5, Number 2.